MATERIALS AND METHODS FOR RAPID AND SENSITIVE DETECTION OF SMALL-MOLECULE TARGETS

GOVERNMENT SUPPORT

This invention was made with government support under 2013-DN-BX-K032 and 2015-R2-CX-0034 awarded by The National Institute of Justice, and DA036821 awarded by National Institutes of Health. The government has certain rights in the invention.

SEQUENCE LISTING

The Sequence Listing for this application is labeled "SeqList-04Apr18-ST25.txt," which was created on Apr. 4, 2018, and is 8 KB. The Sequence Listing is incorporated herein by reference in its entirety.

BACKGROUND

The biosensor field is on a continuous quest for ever-greater sensitivity. In conventional bioassays, where the signal is directly proportional to the target concentration, the sensitivity is determined by the intrinsic target affinity of the bioreceptor being used for detection. In this scenario, it would be difficult to generate a measurable signal at target concentrations more than 100-fold lower than the dissociation constant (K_D) of the bioreceptor. Accordingly, many amplification approaches have been developed in which one binding event can generate multiple signals, allowing detection of targets at very low concentrations.

Enzyme-assisted target recycling (EATR) has proven to be an especially effective way to amplify signals generated from target-binding events. This approach relies on selective, nuclease-mediated degradation of the probe strand of a target-probe duplex that only forms in the presence of the target; this liberates the target, which is 'recycled' for use in additional digestion reactions. Thus, through EATR, a substantial fluorescent signal can be generated by a single copy of the target, thereby greatly decreasing the limit of detection.

Aptamers are nucleic acid-based bio-recognition elements that are isolated in vitro through processes based on systematic evolution of ligands by exponential enrichment (SELEX). Aptamers can bind to a wide variety of targets, including proteins, metal ions, small molecules, and even whole cells. Aptamer-based biosensors have been developed for environmental monitoring, drug detection, and medical diagnostics.

Aptamer-based sensors have many advantages compared to other state-of-the-art methods for the detection of small-molecule targets. Instrumental methods, such as gas or liquid chromatography/mass spectrometry, are sensitive and specific; however, these methods require laborious sample preparation and instruments that are cumbersome and sophisticated, limiting their use for on-site and high-throughput detection. Antibody-based immunoassays, such as ELISA, are highly sensitive and offer high target specificity; however, the in vivo processes for antibody generation are tedious, costly, and challenging, especially for non-immunogenic small molecules. On the other hand, aptamers can be isolated rapidly with controllable affinity and specificity, and are produced without batch-to-batch variation. Additionally, DNA aptamers can be used under harsh conditions and have long shelf lives due to their high chemical stability.

Many strategies have been employed in aptamer-based assays to achieve target detection in an instrument-free manner. In particular, aptamers can be split into two or three fragments that remain separate in the absence of target but assemble upon target binding, and such split-aptamer based sensors have gained popularity as a potential strategy for effective signal reporting.

In principle, the target-induced assembly of split aptamers should be compatible with EATR-mediated signal amplification. Ideally, the split fragments should be highly responsive to target-induced assembly and only the target-aptamer complex should be specifically recognized and digested by the enzyme. However, it is difficult to achieve sensitive target-induced aptamer assembly with conventional split aptamers with a single binding-domain. Small-molecule-binding split aptamers usually have equilibrium dissociation constants (K_D) in the high micromolar range, such that no measurable target-induced aptamer assembly can be observed even with a high concentration of targets. Although target affinity can be improved by engineering split aptamers with longer complementary stems, the majority of thermostable split aptamers undergo some degree of pre-assembly in the absence of target, thereby producing high background signal. Second, in contrast to DNA-based EATR assays, in which target-binding always converts a stem-loop probe structure into a probe-target duplex, aptamer-target binding often gives rise to complex tertiary structures that are non-ideal substrates for nucleases.

Therefore, there is a need for the development of aptamer-based sensors for use in EATR assays. There is also a need for accurate, rapid, sensitive, and powerful means for small-molecule detection by employing EATR assay with aptamer-based sensors.

BRIEF SUMMARY

The subject invention provides methods, assays, and products for detecting small molecules in a complex sample, in particular, in both clinical and field settings. In one embodiment, the method comprises contacting the sample with an aptamer-based sensor selective for a small-molecule target, and detecting the small-molecule target in the sample. Preferably, the aptamer-based sensor is a split aptamer selective for the small-molecule target in the sample.

In one embodiment, the sample is a biological sample of a subject. In a specific embodiment, the biological sample is selected from blood, plasma, urine, tears, and saliva. The subject may be any animal or human, preferably, human. The subjects may also refer to any animal including, but not limited to, non-human primates, rodents, rodents, dogs, cats, horses, cattle, pigs, sheep, goats, chickens, guinea pigs, hamsters and the like.

In one embodiment, the sample is an environmental sample, for example, a water, soil, air, or plant sample. In another embodiment, the sample is a seized drug sample, for instance, a street drug sample seized by law enforcement or government officials.

In one embodiment, the detection of the small-molecule target comprises measuring a signal generated upon assembly of the aptamer-target complex. In another embodiment, the method further comprises determining the concentration of the small-molecule target in the sample.

In one embodiment, the aptamer-based sensor is a cooperative binding split aptamer (CBSA). In one embodiment, the subject invention provides a method for engineering and generating cooperative binding split aptamers (CBSAs) selective for small-molecule targets. CBSAs can be engi-